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Accessory cell functions in mononuclear cell cultures from uremic patients

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Accessory cell functions in mononuclear cell cultures from uremic patients. Mononuclear cells (PBMC) were isolated from dialyzed patients and healthy control subjects. Lymphocyte responses to stimulation with optimal and suboptimal concentrations of lectin (PHA), or stimulation with T cell receptor antibody (Leu 4) were found decreased in the patient cultures. The separate and the combined effects of exogenous interleukin-1 (IL-1) and interleukin-2 (IL-2) were examined in PHA and Leu 4 stimulated cell cultures. Addition of IL-1 did not normalize the decreased proliferation response of the patient cultures. In contrast, addition of IL-2 alone clearly enhanced and almost normalized the response of patient cultures stimulated with suboptimal concentrations of PHA. The combined addition of IL-1 and IL-2 gave no evidence of an additive effect of IL-1 and IL-2. Cell cultures from uremic and normal HLA-identical relative were examined. Substitution of uremic adherent monocytes with normal adherent monocytes as accessory cells did not improve the uremic T cell responses to stimulation with PHA. Furthermore, uremic adherent cells did not suppress the normal T cell responses. These results suggest that uremic accessory cells support T cell activation and, in particular, do not suppress T cell responses. The effect of IL-2 in the present study as well as previous findings of decreased IL-2 production in patients cultures may indicate that uremia primarily influences the proliferation of T cells.

In uremia a decreased immune response has been found in vivo [1, 2] and in vitro [3–6]. In vitro studies have shown that stimulated mononuclear cell cultures from hemodialyzed patients produce less interleukin-2 (IL-2) than normal cell cultures, whereas the expression of IL-2 receptors is unchanged or increased [6]. Activation of T cells is initiated by accessory cells which produce interleukin-1 (IL-1) and present lectins or antigens in the context of class II MHC products. Monocytes/macrophages are the most important accessory cells [7, 8]. Thus, impaired accessory cell function in uremia could be a possible mechanism for the decreased proliferation of uremic lymphocyte cultures. Therefore, the present study was undertaken to examine accessory cell functions in uremic patients.

Methods

Subjects

Peripheral venous blood was obtained before start of hemodialysis from 12 patients (4 females, 8 males) and from 12 (5 females, 7 males) age-matched control subjects. All patients had been on hemodialysis for at least one year and all had received blood transfusion. Predialysis blood samples were also obtained from nine uremic patients (2 females, 7 males) on hemodialysis and their healthy HLA-DR identical and mixed lymphocyte culture (MLC) negative relatives, either siblings or parents prior to kidney donation.

Patients and control subjects had no signs or symptoms of malignant diseases, inflammatory diseases, or were receiving immunosuppressive therapy.

Mitogen and antigen stimulated proliferation

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples as previously described [5, 6].

Triplicates of 5×10^4 PBMCs were cultured in microtiter plates (Nunc, Denmark) in a total volume of 170 μl. Phytohemagglutinin (PHA-P, Difco, Detroit, Michigan, USA) was used as lectin in the cultures in optimal (40 μg/ml culture) and suboptimal (2 μg/ml culture) concentrations. Leu 4 (anti-CD3) was used for direct stimulation of lymphocyte cultures at concentrations with maximal stimulatory effect found by titration (Leu 4; Becton Dickinson, Pennsylvania, USA, 1:20, 20 μl/well). Lectin and Leu 4 stimulated cultures were incubated 72 hours in a humidified atmosphere with 5% carbon dioxide at 37° before 20-hour pulsing of the cultures with ^{14}C-thymidine (^{14}C-thymidine, 20 nCi/well, Amersham, UK) and harvested.

Part of the cultures was in parallel experiment supplemented with 1.25, 2.5 and 5 U/ml culture of purified human interleukin-1 (interleukin-1; Koch Light Ldt., UK) or purified human interleukin-2 (interleukin-2, Electronucleonics Inc., Maryland, USA, 10 μl/well, or approximately 100 U).

Furthermore, six cultures from uremic patients and six controls were supplemented with normal erythrocytes. The erythrocytes were washed three times with saline and added to the culture to a final hematocrit of 1.5% in the culture medium before the PHA stimulation.

Isolation of cells for coculture experiments

Isolated PBMCs from nine patients and nine HLA-identical relatives were used in coculture experiment. Monocytes were obtained by two hour adherence in RPMI 1640 (Gibco) with 10% fetal calf serum (FCS) to plastic culture dishes. The adherent monocytes were detached from plastic after incubation for 30 minutes on ice and rinsed off the plates by ice-cold RPMI-1640 with 10% FCS. The nonadherent cell fraction was

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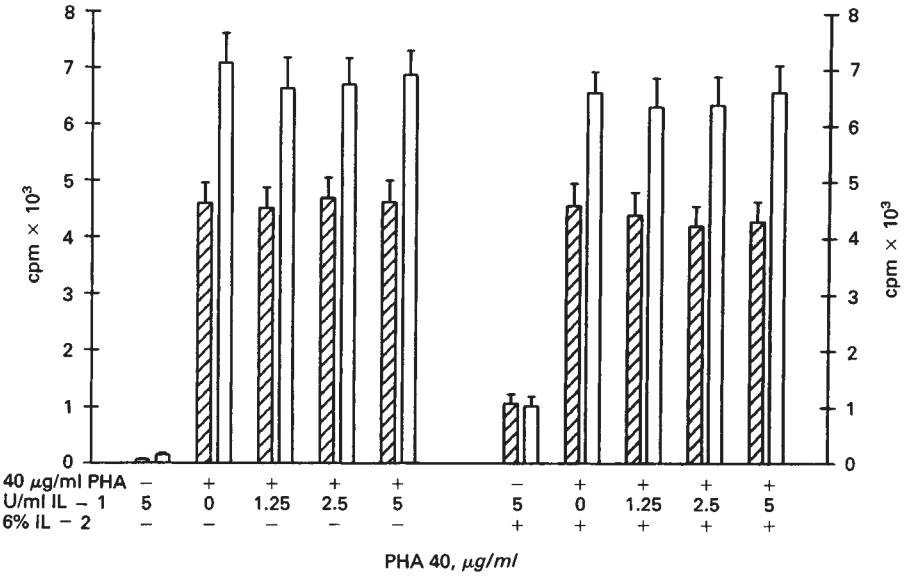

Fig. 1. *Mean ^{14}C-thymidine uptake of 5 × 10^4 peripheral blood mononuclear cells (PBMCs) stimulated with optimal concentrations of PHA (40 μg/ml).* Symbols are (▨) uremic PBMC cultures (N = 12), (□) normal PBMC cultures (N = 12). Vertical lines indicate SEM.

further purified twice over nylon wool columns to obtain a T-cell enriched cell fraction depleted of monocytes/macrophages [9]. T-cell enriched normal and uremic cell isolates (5 × 10^4 cells/well) were then cultured in the presence of 1% and 5% (500 and 2500 cells) normal or uremic adherent cells as accessory cells. As control 2.5 × 10^4 normal and 2.5 × 10^4 uremic PBMCs were cocultured. PHA was used at optimal (40 μg/ml cell culture) and suboptimal (2 μg/ml cell culture) concentrations. The culture conditions were as described above.

Esterase staining of monocytes/macrophages

Esterase staining was done as described by Tucker, Pierre and Jordan 1977 [10]. In one tube six drops of 1 mg% basic fuchsin (T01-0529, Technicon Instruments Corporation, New York, USA) was added to six drops of 7% sodium nitrite (T01-0530) to form the unstable hexazonium pararosanilin. Eighteen drops of 7% sodium cacodylate (T01-0531; buffer and serum lipase inhibitor) were added followed by three drops of alfa-naphtyl butyrat (T01-0680-81) as substrate. In a second tube, two drops of cells and two drops of fetal calf serum were mixed and added. The cells were then fixed for 40 seconds (fixative: 478.5 g diethylenglycol, 50 ml formaldehyde, 10.9 g sodium acetate, and 0.6 g eserin sulphate in 1 liter distilled water). The contents of the second tube was then added to the first tube and incubated for two to three hours at 37°C before microscopic counting of dark red cells.

Statistical analysis

Student's t-test was used for comparison between groups. P values <0.05 were considered significant.

Results

Patient PBMCs stimulated with optimal concentrations of PHA (40 μg/ml) responded significantly lower than the control cultures. IL-1, IL-2, or IL-1 together with IL-2 (Fig. 1) had no additional effect on the response of the control cultures and did not enhance the response of the patient PBMCs. Suboptimal PHA stimulation (2 μg/ml) of the patient PBMC cultures induced lower responses than in the control cultures ($P < 0.05$; Fig. 2). Addition of IL-1 alone improved patient as well as control PBMC responses, but the effect did not reach significance. In contrast, addition of IL-2 clearly enhanced both control and patient culture proliferation ($P < 0.01$) and decreased the difference between the two responses. No additive effects of IL-2 and IL-1 were observed. Results of stimulation with Leu 4 alone (Fig. 3) were comparable with those of suboptimal PHA stimulation. IL-1 alone had no effect on patient and control cultures, whereas IL-2 improved the PBMC responses ($P < 0.05$) in both uremic and control cultures. The PHA-2 and Leu 4 responses of uremic cell cultures with exogenous IL-2 were not significantly different from the normal cell cultures without exogenous IL-2 ($P > 0.1$).

Figure 4 shows the results of addition of erythrocytes to the culture medium. Addition of erythrocytes to the cultures improved the mitogen responses of patient and control cultures by about 20%, but did not normalize the decreased responses of the uremic cell cultures. The percentage of esterase positive cells (macrophages) was similar in patient and control PBMC cultures (11.6 ± 1.1% [SEM], and 13.3 ± 1.5%, N = 11, $P > 0.05$). In the coculture experiments, the mixed lymphocyte culture (MLC) responses were 1% of those obtained by PHA-stimulation, confirming the HLA-D identity between the nine patients and the nine healthy donors [11]. Stimulation of the patient and control PBMC cultures with 40 or 2 μg/ml PHA showed slightly lower responses of the uremic cell cultures, but the difference did not reach significance in this small material. However, there was a significant difference in the responses to 2 μg/ml PHA in the T-cell enriched fractions when normal and uremic patient cultures were compared (Fig. 5). Five percent adherent cells from patients or controls equally well enhanced T cell proliferation at suboptimal PHA concentrations (2 μg/ml, Fig. 5) and the same pattern was seen with PHA 40, but significant only for normal T cells (Fig. 6). Furthermore, there was no evidence that uremic adherent cells suppressed normal T cell responses to PHA, and no suppressive effect of uremic

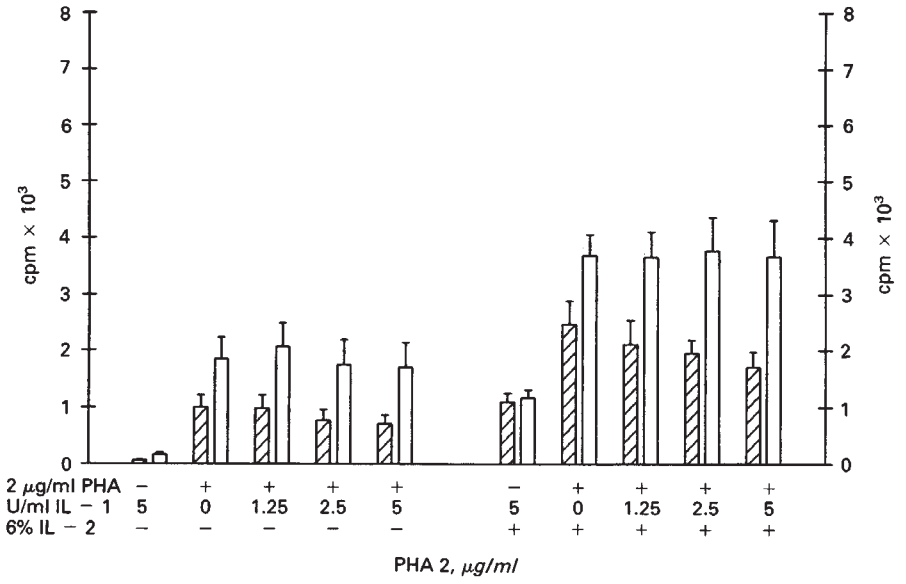

Fig. 2. *Mean ^{14}C-thymidine uptake of 5 × 10^4 peripheral blood mononuclear cells (PBMCs) stimulated with suboptimal concentrations of PHA (2 μg/ml).* Symbols are: (▨) uremic PBMC cultures (N = 11); (□) normal PBMC cultures (N = 11). Vertical lines indicate SEM.

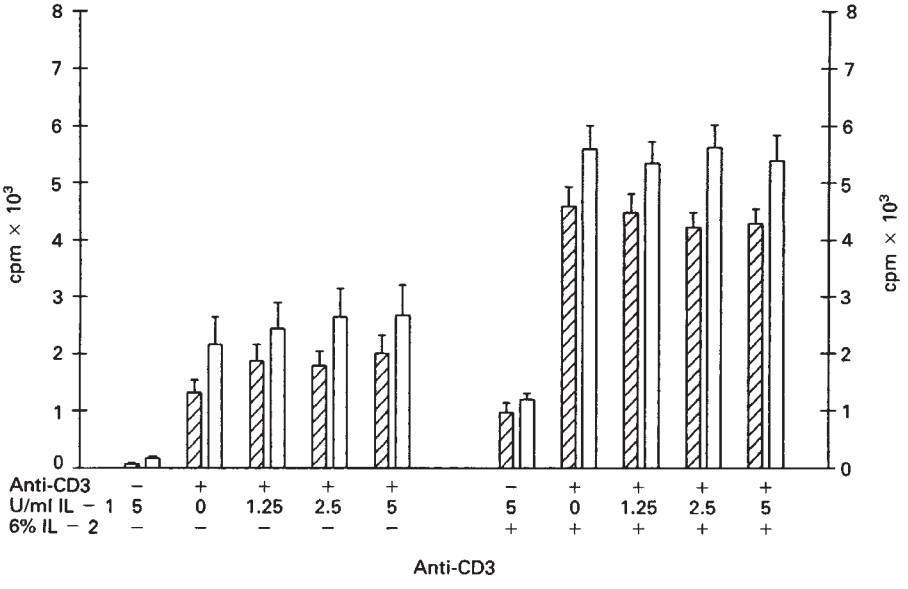

Fig. 3. *Mean ^{14}C-thymidine uptake of 5 × 10^4 peripheral blood mononuclear cells (PBMCs) stimulated with optimal concentrations of Leu 4 (20 μl, 1:20).* Symbols are: (▨) uremic PBMC cultures (N = 12); (□) normal PBMC cultures (N = 12). Vertical lines indicate SEM.

bulk PBMCs could be demonstrated when cocultured with normal bulk PBMCs.

Discussion

Previous studies [4, 5] have shown that PBMC cultures isolated from uremic patients respond lower than normal cultures to lectin stimulation as measured by DNA synthesis and production of IL-2. Expression of receptors for IL-2 has, however, been found normal in PHA-stimulated lymphocyte cultures from uremic patients [6] and cytotoxic effector cell functions that do not require cell proliferation remain nearly normal in uremia [12]. Antigen responses have also been found low [3, 13]. These findings of both in vivo and in vitro decreased antigen responses [14] suggest that accessory cell functions which initialize activation of T cells could be impaired. One mechanism could be a decreased production of IL-1 by the accessory cells. This could eventually result in a decreased proliferation of activated T cells and a lower production of IL-2 as previously found in uremic cell cultures [4–6]. In this study we take advantage of purified preparations of IL-1 and IL-2 which have recently become available. We have furthermore studied accessory cell functions in coculture experiments of HLA-identical cell combinations. However, the present results give little support for a monocyte/macrophage defect. First, IL-1 did not reconstitute the patient cell responses whereas addition of exogenous IL-2 significantly improved the uremic cell responses. Second, coculture experiment showed that uremic macrophages/monocytes have the same capacity to serve as accessory cells for suboptimally stimulated T cells (Fig. 5). In these experiments a MLC stimulatory effect could be excluded since HLA-identical MLC negative combinations were used. Third, the percentage of esterase positive cells in the

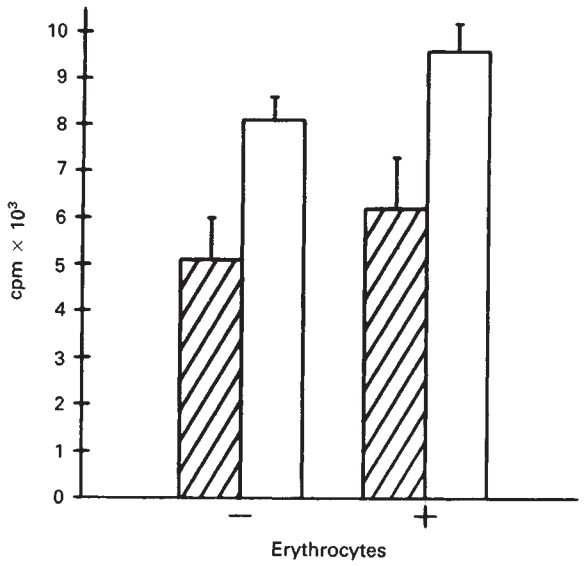

Fig. 4. *Effect of ^{14}C-thymidine uptake with or without addition of erythrocytes to uremic and normal peripheral lymphocytes cultures stimulated with PHA (40 μg/ml).* Symbols are: (▨) uremia; (□) control. The difference between uremic and normals is significant ($P < 0.001$).

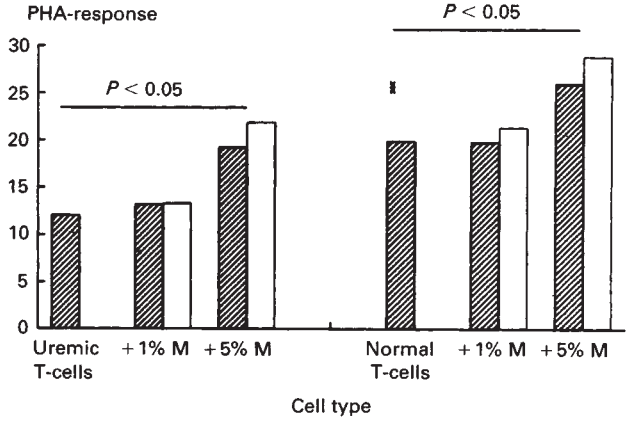

Fig. 5. *Mean PHA (2 μg/ml) responses (^{14}C-thymidine uptake) in cocultures of isolated T cells from uremic patients with macrophages from their healthy HLA-D identical relatives, and vice versa.* Hatched columns denote T cells alone or with 1% or 5% uremic macrophages (M), open columns are T cells supplied with normal macrophages. *$P <$ 0.01 vs. uremic.

patient and the control PBMC cultures were similar and in accordance with the percentage published with this technique by Tucker, Pierre and Jordan [10].

Decreased in vitro transformation of uremic lymphocytes has in some studies been attributed to an enhanced suppressor activity by adherent cells (monocytes/macrophages) which are the predominating prostaglandin secreting cells [15]. In humans the results have been conflicting [16, 17], but in uremic rat models an enhanced suppressor activity by adherent cells has been reported [18]. Prostaglandins produced by macrophages have been shown to suppress T cell proliferation [19] and IL-2 production [20], but in the present study macrophages from patients showed no suppressive effect on the normal T-cell

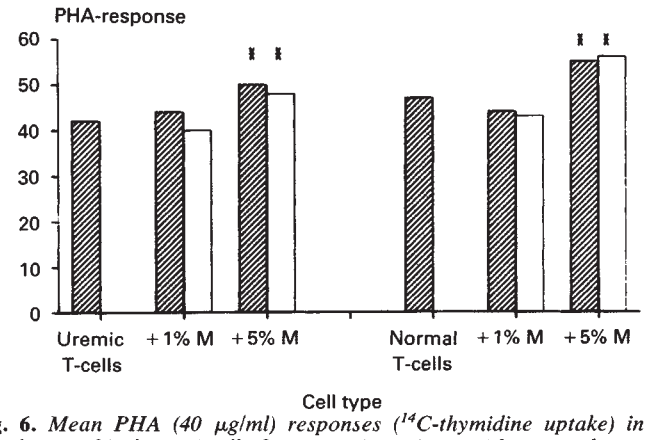

Fig. 6. *Mean PHA (40 μg/ml) responses (^{14}C-thymidine uptake) in cocultures of isolates T cells from uremic patients with macrophages from their healthy HLA-D identical relatives, and vice versa.* Symbols are identical to Figure 5. *$P < 0.05$.

responses, and previous studies [6] have shown that blocking of PGE_2 production by indomethacin does not normalize the uremic lymphocyte responses. It cannot, however, be excluded that a suppressive effect of macrophages may be more pronounced in other lectin systems.

We cannot rule out that functions of other accessory cells are impaired in uremia. Dendritic cells (DC) are known to be active accessory cells in mice and in humans [7, 21, 22]. DC are plastic non-adherent cells and are therefore excluded in the present study by the plastic adherence procedure of the monocytes/macrophages.

In rare conditions impaired in vitro lymphocyte proliferation is caused by a deficiency of the enzyme system needed for the anaerobic glycolysis. This can be reversed by addition of an exogenous oxygen source [23, 24]. Addition of normal erythrocytes as oxygen suppliers improved the mitogen response of uremic and normal lymphocytes equally well, which show that the anaerobic condition that prevails in most culture medias does not especially injure uremic cells.

This and previous studies [5, 12] of immune responses in uremia confirm that the proliferative capacity of uremic T cells is impaired. Accessory cell functions as well as cytotoxic effector cell functions, however, seem normal.

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